

RESEARCH HIGHLIGHT

Brain Cytochrome p450 enzymes: a possible Therapeutic Targets for Neurological Diseases

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Neurological diseases (ND) afflict hundreds of millions of people worldwide and constitute 12% of total deaths of the population all around the world. According to a global study conducted by the World Health Organization, 8 out of 10 disorders in the 3rd highest disability classes are neurologic problems. Commonly they have disabling consequences, compromising severely the quality of the life of affected subjects. Often therapeutic strategies are limited in efficacy. Limitations lie with the enormous fragility of the so complex structure and functions accomplished by the central nervous system, together with the presence of the barrier between the tissue and the blood limiting the penetration of drugs. For these reason new strategies to protect the brain are essential. One way might be the enhancement of local biomolecular mechanisms (metabolizing enzymes, transporters, etc.). However, increasing evidence underline that our knowledge of the brain is still partial, and information cannot simply be derived from other well-known organs such as the liver. This is the case for the brain cytochrome P450 enzymes, showing differential cellular localization, brain regional expression and modulation.

Keywords: Brain; cytochrome P450 mono-oxygenases; differential modulation; xenobiotic metabolism; therapeutical targets; Bilirubin toxicity; Parkinson's disease

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Neurological diseases (ND) afflict hundreds of millions of people worldwide and constitute 12% of total deaths of the population all around the world ^[1].

Cytochrome P450 enzymes (Cyps) are a heme-containing superfamily of detoxifying enzymes, responsible also for the bio activation of a large spectrum of endogenous molecules and xenobiotic such as antibiotics, steroid, fatty acids, vitamins, among others ^[2-4]. Largely present in liver, they are erroneously considered expressed low in the CNS (1-5% of liver) ^[5]. Consistent with the enormous heterogeneity of the

brain (in cell type, distribution, functions, architecture, etc.), bCyps levels are cell/region specific ^[3], and their localized expression may reach levels similar or higher than in the hepatocytes ^[6-8]. In addition, it is recognized that different, specific Cyps genes are expressed in the neural tissue, and that their regulation should differ from the modulation in peripheral tissue. For their particular characteristics, brain Cyps sustain essential neurotrophic and neuroprotective functions, regulate the neurotransmitters level (such as the endogenous GABA-A receptor agonists), maintain the cholesterol and the cerebral blood flow homeostasis, act on

Table 1. brain Cyps's substrates and their association with neurological pathologies

CYP	Physiological substrate	Substrate pharmacological drug	“environmental” substrate	Related pathology
1A1	Melatonin, Estradiol, Arachidonic acid, Progesterone, Testosterone, Prostaglandin H2, Bilirubin	Phenobarbital, BNF, Tamoxifen, Chloroxazone, 3-methylcholanthrene	Benzo(a)pyrene, TCDD, MCA, theophylline	Chemical carcinogenesis, Cancer
1A2	Melatonin, Arachidonic acid, Cholesterol, Steroids, Bilirubin	Clomipramine, Clozapine, Melatonin, Phenobarbital, β NF, Acetaminophen, Duloxetine, Amitriptyline	TCDD, MCA, Caffeine, Aflatoxin B1	Chemical carcinogenesis, Cancer
2B6	17- β estradiol, Anandamide, Arachidonic acid, Estrone, Serotonin, Testosterone	Bupropion, Diazepam, Ketamine, Methadone, Meperidine, Efavirenz, Pentobarbital, Phencyclidine, Propofol, Sertraline, Selegiline, Ifosphamide, Tramadol, Cyclophosphamide	3,4-methylenedioxy-amphetamine (ecstasy), Nicotine, Chlorpyrifos, DEET (Insecticides), Malathion, Paraquat, Parathion, Cacao's Anandamide	Ethanol brain damage.
2D6	5-methoxytryptamine, Anandamide, Progesterone, Tyramine, Tryptamine, Octopamina, Synephrine, Testosterone, Allopregnenolone 17 α -hydroxyprogesterone, 5 α -Dihydroxyprogesterone	Amyltriptyline, Brofaromine, Clomipramine, Codeine, Fluoxetine, Citalopram, Clozapine, Desipramine, Tamoxifen, Dextromethorphan, Ethylmorphine, Fluvoxamine, Haloperidol, Hydrocodone, Imipramine, Mianserin, Mirtazapine, Nicergoline, Nortryptaline, Oxycodone, Paroxetine, Perphenazine, Risperidone, Tramadol, Levodopa, Tranylcypromine, Venlafaxine, Zuclopenthixol	MPTP (pesticides), Parathion, Nicotine	Nicotine linked learning and memory defects, and PD protection. Pesticide linked PD risk. Anxiety. Impulsivity. Schizophrenia drugs reduced efficacy. Smoke linked mental decline.
2E1	17- β estradiol, Arachidonic acid, Estrone, Prostaglandin, Dopamine	Enflurane, Elbamate, Halothane, Soffurane, Sevoflurane, Trimethadione, Acetaminophen	Acetone, Aniline, Nicotine; Benzene, Carbon tetrachloride, Chloroform, Ethanol, Chlozoxazone, Phenol, Theophylline, Trichloroethane	Glioblastomas, Anaplastic astrocytomas, Ethanol brain damage. Smoke linked mental decline.
3A4	Testosterone, Androstenedione, DHEA, Progesterone, Sterols	Acetaminophen, Codeine, Cyclosporin, Diazepam, Erythromycin, Phenobarbital, Delavirdine, Amitriptyline, Citalopram	TCDD, caffeine, cocaine	Mental and reproductive disorders linked to frontal lobe epilepsy drugs.

Numbers indicate the different brain Cyps. TDCC:2,3,7,8-tetraclorodibenzo-p-diossine; MCA: 3-methylcholanthrene; NF: beta-Naphtoflavone; DEET = N,N-diethyl-m-toluamide; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; DHEA: Dehydroepiandrosterone; PD: Parkinson's Disease.

retinoid clearance and control the temperature [9;10]. Moreover, they act in drugs or chemicals activation/degradation and the development of specific neurological diseases (ND) [11] (Table 1).

For instance, Cyp2D7 that metabolizes codeine to morphine, a potent analgesic, is present in brain with an alternative splicing not present in liver [12]. Cyp2A3 is induced by -naphthoflavone in liver, but not in astrocytes cells [13]. Similarly, smoke induces both brain, but not hepatic, CYP2D6 [14] and CYP2B6 [15]. Moreover, brain CYP

modulation may be strictly region and cell specific, influencing, or even causing, pathological consequences. This is the case of nicotine and ethanol induction of brain CYP2D6 (Fig 1). Nicotine induces the enzyme in the substantia nigra, putamen, brainstem, and only in selected cells of the cerebellum, frontal cortex and hippocampus [16]. This upregulation negatively affects learning and memory by one side [17;18] by the other side, the CYP2D6 induction protects against Parkinson's disease (PD), and extends the effect of levodopa, a common drug for treatment in PD [16]. On the contrary, the exposure to pesticides together with

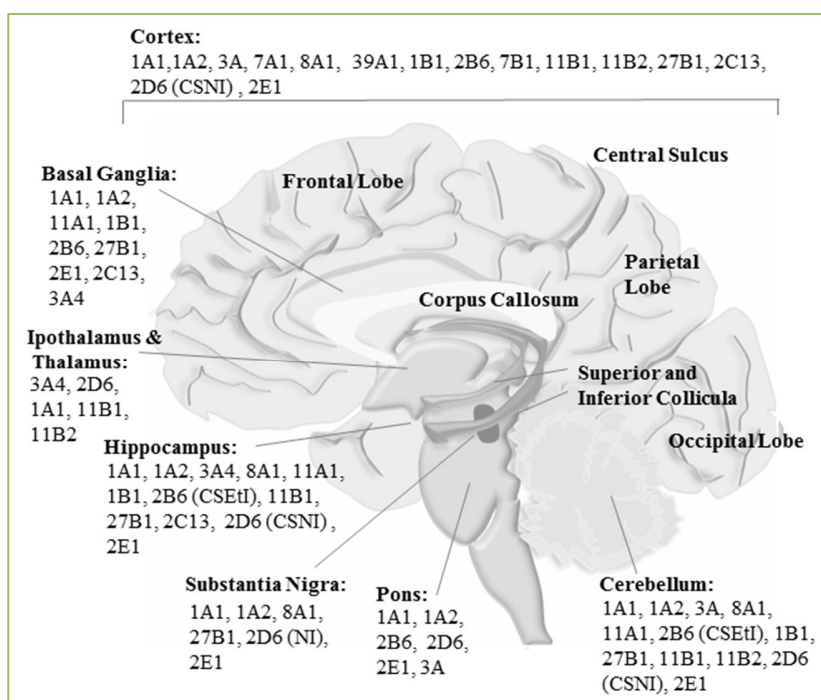


Figure 1. brain Cyps expression and specific induction in the CNS. Numbers indicate the different brain Cyps. NI: Nicotine Induced; CSNI: Cell Specifically Nicotine Induced. CSEtI: Cell Specifically Induced by Ethanol.

reduced CYP2D6 activity increases the recurrence of PD [19,20]. Similarly, poor metabolizers (due to CYP2D6 genetic variants) suffer for increased anxiety and impulsivity [21;22], which could be justified by lower levels of endogenous dopamine or 5 ôhydroxytryptamine; it has not been purposely declared if that could be explained by lower receptor levels, justified by genetic predisposition or due to the previously stated CYP2D6 variants [9]. Schizophrenic patients if are Cyp2D6 poor metabolizers may suffer from lack of efficacy when treated with clozapine, aripiprazole, olanzapine and thioridazin, all substrates for the enzyme [21,23]. Ethanol specifically induces CYB2D6 in CA1-3 pyramidal cells and dentate gyrus granular neurons (hippocampus) and Purkinje cells (cerebellum). These cells/brain areas are known to be damaged by alcohol [24]. Cyp3A4 in hippocampus is induced by drugs used for the temporal lobe epilepsy, with possible side effects such as mental and reproductive disorders, acting on sexual hormone metabolism [25].

With respect to the above mentioned these underlines three essential points. First, the relevance of brain Cyps in CNS homeostasis, second their association in the ND and third, the potential to use them as therapeutic targets for neuroprotection.

Taking into account these considerations, recently we assayed the use specific brain Cyps (Cyp1A1, 1A2, 2A3=2A6 in human) to confer *in situ* (CNS) resistance to

bilirubin, an endogenous potentially neurotoxic molecule displaying a strong topography of damage (cerebellum the most damaged area) [13]. The Cyp1A and 2A subfamily are largely expressed in liver, where they are able to metabolize bilirubin when hepatic glucuronidation is deficient, reducing blood concentration and consequently decreasing the risk of developing neurological consequences [26-29]. The same Cyps are also expressed in neurons and glial cells, and most important, prone to be modulated in astrocytes [8;30] making them a potential therapeutical tool. To upregulate the Cyps expression and increase the bilirubin clearance, we used a powerful hepatic Cyps inducer (-naphthoflavone - BNF). This approach allowed us to prove the dual role of bilirubin as brain Cyps inducer and substrate, and to improve bilirubin clearance and increase cell viability [13]. However, the behavior (mRNA expression, activity, mechanisms of action) of the brain Cyps we analyzed was strongly different among different Cyps (Cyp1A1, 1A2 and 2A3) and region specific. First, differently from hepatic Cyps, Cyp2A3 was not modulated by BNF, and thus barely efficient in the metabolite clearance. Cyp1A1, was upregulated in both cortex (after 6H from BNF exposure) and cerebellum astrocytes (after 24H), and effective in bilirubin oxidation, but only after uncoupling by the ôpollutantsö 3, 4, 3,4-tetrachlorobiphenyl (TCB). As proposed by Schelzinger and Zaccaro, the uncoupling of Cyps from the respiratory chain increase the reactive oxygen species (ROS) and in turn oxidize bilirubin [27,31]. Despite oxidative stress is usually

considered part of the damaging mechanisms, ROS production was unexpectedly not only tolerated but even beneficial (improving viability) in our model. What is more, we identified the Cyp1A2 as the most relevant brain Cyps in lowering the bilirubin cell concentration without need of uncoupling [4,32]. Unfortunately, an appropriate Cyp1A2 modulation was reached only in cerebral cortex (24H), confirming again that brain Cyps modulation is region, cell type and inducer specific.

What mentioned before outline the need of a better understanding of how to achieve a region-specific modulation of the target genes to uses it as a possible therapy. However, more and more evidence underline that our knowledge of the brain is still partial, and information cannot simply be derived from peripheral organs (such as liver), it needs to be evaluated specifically in the CNS for each pathology.

The difference in timing and extent of bCyp modulation between cortex and cerebellum we observed may be explained in several ways. First, most of CYPs are regulated by nuclear receptors bearing to the AhR (aryl hydrocarbon receptor), CAR (constitutive androstane receptor), PXR (pregnane X receptor) and RXR (retinoid X receptor) families (reviewed in [33]). Due to the complexity of the CNS, they should be differentially (region/cell type specifically) expressed [34-38], making bCYPs in one region more prone to induction than in another area. Second, and more specifically for our study, the differential behavior may be due to a different maturational stage of the two regions [39,40] which again could influence the level of expression (thus the activity) of the pathway involved in bCyp modulation [41,42]. Third, strictly looking at the therapeutic goal, different metabolic kinetics might be explained also by the presence of mRNA splicing variant influencing the substrate specificity [43,44].

The existence of the blood brain barrier may limit the availability/efficacy of the inducer(s). To overcome this issues, a more detailed knowledge of the signaling pathway available (to modulation) in each area of the brain must be acquired. Due to their essential role in brain homeostasis, the subtle modulation of bCyps activity should be limited in time, allowing the reversal of the effect to the physiological activity in order to limit the potential side effects [14].

Overall, our data emphasize the increasing importance of knowledge about bCyps modulation in pharmacological and toxicological implications for cerebral drug metabolism, in order to use them as an important tool to increase brain resistance to neurological diseases.

Conflict of Interest:

This statement is to declare that there are not conflicts of interest about this *Ms.* and that the supporting agencies did not play any role in the study. The *Ms.* has been read and approved by all the authors. The material has never been published, and is not under consideration elsewhere.

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